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SUBJECT OF INVESTIGATION

HISTOCHEMICAL STUDIES ON THE DISTRIBUTION OF ENZYMES, ESPECIALLY OXIDASES AND PHOSPHATASES IN THE LIVING BODY

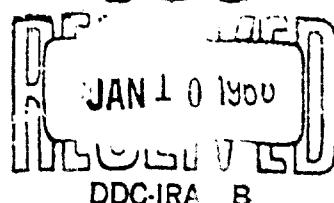
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Semifinal Report

DA-CRD-AG-S92-544-64-G23

29 July 1964 to  
28 Jan. 1965

Histochemical studies on the distribution of  
enzymes, especially oxidases and phosphatases  
in the living body

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Histochemical studies on the distribution  
of enzymes, especially oxidases and  
phosphatases in the living body

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## A B S T R A C T

Two giant salamanders, *Megalobatrachus japonicus* were used for materials.

The blood was carefully taken from the tail and smear preparations were obtained. The blood smears were fixed one minute with ethanol-formol (9:1) solution prior to staining, washed thoroughly in distilled water, and stained five minutes with either of the following peroxidase reagents freshly prepared each time.

1) Mixture of benzidine 0.3 gr., 3%  $H_2O_2$  10 drops, and distilled water 100 cc. This mixture should be filtered because of poor solubility of benzidine in water.

2) Mixture of orthophenylenediamine 0.1 gr., 5%  $H_2O_2$  10 drops, and distilled water 100 cc.

The smears were then washed in water, and counterstained with dilute G i e m s a stain for ten to fifteen minutes.

Finally they were washed in water, and exposed to air till dry.

The alkaline phosphatase technic ( modified technic of the lead sulphide method of T a k e u c h i) was also applied for blood staining.

The preparation stained with the benzidine peroxidase reagent, that with the orthophenylenediamine reagent, and that with G i e m s a stain, were simultaneously observed, and compared.

In the giant salamander, the neutrophil leukocytes generally showed peroxidase activity, while the eosinophil and basophil leukocytes did not. The reaction was lacking in all cells of the lymphatic series. As is well known, the general feature of the peroxidase reaction in human leukocytes under the light microscope is that the cytoplasm is diffusely, fine granularly stained except eosinophil leukocytes in which the reaction may take a pattern of prominent coarse granules corresponding to the eosinophil granules.

A gigantic peroxidase granule was detected in the polymorphonuclear neutrophil leukocytes of the giant salamander, *Megalobatrachus japonicus*. Among 70 different animals examined, this gigantic peroxidase granule was found only in the giant salamander in apparently healthy condition. This granule was generally localized in the center of the leukocyte, single in number, six to ten  $\mu$  in diameter, and could be demonstrated both with the benzidine peroxidase

reagent and the orthophenylenediamine peroxidase reagent.

This feature resembles the gigantism of the peroxidase granule that was recognized in the Chediak (1952) and Higashi's disease (1954). This congenital human disease is characterized by albinismus with photophobia, early death of albinic sibs and peculiar pathological blood picture in which the above-mentioned gigantic granules or inclusion bodies are demonstrated in the leukocytes. In this disease the peroxidase granules gather in a few or several spots to form round clumps or globes not only in the neutrophils but also in the monocytes, eosinophils, myelocytes and the myeloblasts, in other words it is an abnormality affecting all the cells of the myelogenous system.

It is of interest that one of pathological characters of human leukocytes was found in an apparently healthy giant salamander, although it remains to be determined in what way this gigantic peroxidase granule developed only in the neutrophil leukocytes of this animal.

The gigantic peroxidase granule in the giant salamander may be derived from a conglomeration of the peroxidase substance within the leukocyte under abnormal or normal metabolism of protein and other substances in

this animal, or may be a product caused by phagocytosis of a peroxidase reacting substance. However, if a phagocytosis phenomenon is assumed in the giant salamander, it remains still unanswered why it occurred only in the neutrophil leukocytes and not at all in other leukocytes such as monocytes.

It is of special interest that peculiar and significant findings in blood cells of man and animals may frequently be obtained with the aid of these cytochemical reactions.